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Investigation of lipid oxidation in the raw materials of a topical skin formulation: A topical skin formulation containing a high lipid content.

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Abstract

Several studies have demonstrated that lipid oxidation often occurs in topical skin formulations which can affect product odour (both positively and negatively). Furthermore, odour detection threshold values and odour descriptors of identified volatile oxidation products in cleansing and skin cream formulation prototypes were recently determined by a trained sensory panel at the Technical University of Denmark in the Division of Food Technology. In this study, we investigated lipid oxidation in a prototype skin cream formulation as well as in selected cosmetic skin care raw materials. Lipid oxidation was measured regularly over a six-month period for the product and over a three-month period for the raw materials by headspace gas chromatography–mass spectrometry. The volatile compound present in the highest initial concentration, and which increased most during storage, was 3-methyl-1-butanol (medicinal, chemical/cleaning agent odour), and its formation was linked to the raw material isoamyl p-methoxycinnamate. The odour character of the product after storage was assessed and informally deemed acceptable for consumer usage and typical of topical dermocosmetic products. A potential pathway for its formation was also identified. In addition, the concentrations of several well-known lipid oxidation products increased during storage and were suggested to originate primarily from rice bran wax, which oxidized more readily than other raw materials due to its unsaturated nature.

Introduction

Several studies have shown that lipid oxidation often occurs in topical skin care formulations containing unsaturated lipids and that lipid oxidation products can affect product quality (1–6) (i.e. odour (4–6) and colour (2)), potentially impacting product both positively and/or negatively.

Earlier studies have shown that raw materials were at least partly responsible for volatile compounds present in simple emulsions immediately after their production (7,8). Since topical skin care formulations are often emulsions, knowledge obtained from studies on simple emulsions can provide some understanding of the mechanisms behind the formation of volatile compounds in topical products. However, the composition of topical skin care formulations is far more complex than that of simple emulsion systems and so are the oxidation mechanisms. In order to determine whether/which raw materials are responsible for volatile compounds present in freshly produced topical skin formulations, several factors must be considered: volatiles introduced by raw materials, production method (e.g. temperature and other processing conditions as well as exposure to oxygen and light), and the mechanisms leading to the formation of volatile compounds (7–12). Volatile lipid oxidation products can also be formed during storage as a result of interactions between raw materials, production method and storage conditions. Temperature and exposure to oxygen and light during storage are factors that can influence the rate of lipid oxidation after production.

Other studies have investigated the effect of impurities in raw materials on oxidative stability in finished food products and model emulsions, as summarised in a review by Waraho *et al.* (12), who concluded that the oxidative stability of the finished product was linked to the quality of the raw materials. Since some raw materials used in foods are common with cosmetics, studies performed on raw materials for food can be used as guidance for cosmetics.

In a study on raw materials for personal care products, the impact of the production method on the quality of myristyl myristate, a skin conditioning and opacifying agent, was explored (13). The purity of myristyl myristate products varied from 80.1% to 97.5% between manufacturers. Furthermore, the oxidative status of the myristyl myristate products measured by peroxide value (PV) fluctuated from <0.1 to 6.0 meq/kg depending on the manufacturer and product grade. In addition, the colour, acid value (0.2 - 0.8 mg/g), hydroxyl value (1.6 - 14.0 mg/g) and saponification value (128 – 134 mg/g) also varied widely between the production methods used (13).

Two other studies investigated the oxidative stability of skin creams with new active ingredients, and both studies showed significant changes in physical and oxidative stability as well as odour properties as a result of the addition of extracts from Icelandic brown algae *Fucus vesiculosus* (2,14). This highlights the importance of securing each raw material´s quality, stability and an understanding of raw material interactions.

The aim of this study was to explore lipid oxidation in selected raw materials and in a topical skin formulation containing high levels of lipids. A second aim was to correlate any raw material oxidation

with the finished product oxidation to identify any culpable agents. In addition, we aimed to understand the mechanism leading to the formation of any identified volatile compounds.

Materials

Prototype Skin Cream Formulation (PSCF)

The prototype skin cream formulation was produced by GlaxoSmithKline (Brentford, United Kingdom) and contained several raw materials including rice bran wax, glycerine, isostearyl isostearate, palmitic acid monoethanolamine (PMEA). The prototype skin cream formulation contained approximately 29 % of lipid.

Raw materials

Separately to the aforementioned prototype product, individual (cosmetic-industry-relevant) raw materials were assessed for lipid oxidation potential:

- Rice bran wax (Koster Keunen, Bladel, Netherlands),
- Glycerine (Croda Europe Ltd, East Yorkshire, England),
- Isostearyl isostearate (Croda Europe Ltd, East Yorkshire, England),
- Palmitic Acid Monoethanolamine (PMEA; Jan Dekker, Wormerveer, Netherlands),
- Isoamyl p-methoxycinnamate (UV cinnamate) (Symrise AG, Holzminden, Germany),
- Bis-ethylhexyloxyphenol methoxyphenyl triazine (UV triazine) (BASF SE, Ludwigshafen, Germany),
- Hexyl 2-(1-(diethylaminohydroxyphenyl)methanoyl)benzoate (UV benzoate) (BASF SE, Ludwigshafen, Germany).

Methods

Storage conditions

PSCF was stored for 6 months at 5ºC, 20ºC and 40ºC without exposure to light and at 20ºC with exposure to light and for 2 weeks at 50ºC. Samples were taken after 0, ½, 1, 2, 3 and 6 months.

Raw materials were stored at 40ºC for 3 months; samples were taken after 0, 1, 2 and 3 months of storage.

The samples were stored in closed 40 ml opaque bottles. Samples were stored in individual bottles, to be withdrawn at each time point for each analysis. After sampling, all samples were stored at 5ºC until analysis.

Oil extraction methodology

Oil was extracted from 5 g of PSCF and UV cinnamate with the Bligh and Dyer method (15) (n = 2). However, a reduced amount of solvent was applied as described by Iverson *et al*. (16). In brief, lipids were extracted by the use of a homogenous mixture of 20 ml of chloroform, 20 ml of methanol and 15 ml of water. The water soluble parts were separated from the lipid soluble parts by a subsequent addition of chloroform and methanol. Phase separation was completed by centrifugation. After phase separation was completed, chloroform in the chloroform and lipid phase was evaporated, and the oil content could then be determined gravimetrically. The lipid extract was used as the starting material for analysis of PV and determination of fatty acid composition.

Determination of Peroxide Value

PV was measured using the IDF method (17) and quantified by colorimetric determination of iron thiocyanate spectrophotometrically at 500 nm by UV mini 1240 (Shimadzu, Duisburg, Germany) (n = 2). The spectrophotometer was reset to detect chloroform/methanol (7:3) solvent as zero.

Quantification of volatile compounds

Extraction of volatile compounds, GC-MS analyses and quantification were done automatically as described by Thomsen *et al.* (18) with the following modification of the sample preparation, collection and water evaporation (Table 1). These modifications were done in order to extract volatile compounds from all matrices, to avoid contamination of the tube by powders and to remove water residues.

Briefly, volatile compounds were collected from 1 g of sample in a 10 mL vial (n = 3). The automation sequence was: incubation for 4 min at a temperature of 60 °C or 45 °C (see Table 1). The sample was agitated at 300 rpm (agitator on time: 10 s, agitator off time: 1 s). Thereafter, purging with nitrogen at 50 ml/min through the headspace of the vial was started for 20 min. The volatile compounds were trapped on tubes containing Tenax GR 300 (Gerstel GmbH & Co. KG., Mülheim an der Ruhr, Germany). Water residues were removed from the tubes with a 50 mL/min purge flow (see Table 1). Then the volatile compounds were desorbed from tubes in the thermal desorption unit (initial temp 40 °C, then 720 °C/min to 280 °C kept there for 5 min) to the GC. The volatile compounds were analysed

on a GC-MS model: HP 6890 - HP 5973 (Agilent Technologies, USA). Chromatographic separation was performed on a DB1701 column (30 m × ID 0.25 mm × 0.5 µm film thickness, J&W Scientific, Folsom, CA, USA) using helium gas flow (1.3 mL/min) in the GC. The MS settings were: 70 eV, electron ionization mode, mass to charge ratio (m/z) scan between 30 and 250. The GC temperature-program was as follows: initial 45°C, 5°C/min until 90°C, 4°C/min to 220°C and held for 4 min.

Fatty acid methyl esters (FAME)

Fatty acid compositions in oil and Bligh and Dyer extracts were determined as described by Safafar *et al*. (19) (n = 2). In brief, 1 g of Bligh and Dyer extract or 0.3 g of oil were weighed in test tubes. The chloroform was evaporated from Bligh and Dyer extract with nitrogen. Then, internal standard 23:0 was added to the oil and extracted together with heptane with BHT, toluene and borontrifluoride in methanol. Samples were mixed and methylated in a microwave oven (Microwave 3000 SOLV, Anton Paar, Ashland, VA, USA) and then cooled down. Saturated NaCl and heptane with BHT were added and thereafter phase separation occurred. The upper phase of the sample was transferred into 1 mL vials and analysed by Agilent 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a DB-WAX fused silica capillary column (10 m×0.1 mm, 0.1 µm; Agilent Technologies, Palo Alto, CA, USA), helium as carrier gas and a flame ionization detector. The GC temperature program: initial 160 °C, 10.6 °C/min until 200°C and held for 0.3 min, 10.6°C/min to 220°C and held for 1 min, and 10.6°C/min to 240°C and held for 3.8 min. Fatty acids were identified by comparing their retention time to that of authentic standards. Fatty acids were expressed as % fatty acid of total fatty acids from C8-C24.

pH determination

The pH was measured using a Metrohm 827 pH meter (Metrohm, Herisau, Switzerland).

Description of difference scale

An expert panel of 3 scientists conducted a fast industry standard method to assess the odour changes. In this method, the sample odour was graded versus a reference sample stored at 5°C. The samples were ranked from one to five based on a scale description of difference (DOD) between sample and reference sample (Table 2). All samples ranked three or less were deemed within product range.

Statistical analysis

A two-way analysis of variance and a Bonferroni multiple comparison test were employed to evaluate significant changes in Figure 1 and 2. The significance level was 0.05. The statistical analysis was conducted using Graph Pad Prism version 6 (Graph Pad, La Jolla, USA).

Results and discussion

Lipid oxidation in PSCF: PV and volatile analysis

PV was used as a measurement of the primary oxidation products, lipid hydroperoxides. PV was initially 0.62 ± 0.01 meq/kg and remained below 0.65 meq/kg during the 6 months of storage at 5°C, 20°C and 40°C (data not shown). When exposed to light during storage, the PV increased slightly to 1.44 ± 0.17 meq/kg. According to PV, lipid oxidation only occurred to a low extent. However, a low PV does not necessarily imply that no oxidation has occurred; it may be related to rapid conversion of lipid hydroperoxides to secondary volatile oxidation products. It is therefore also advisable to assay for secondary lipid oxidation products.

The assay for secondary volatile oxidation products, via dynamic headspace GC-MS analysis, confirmed that the low PV was due to a fast conversion to aldehydes and alcohols. The concentration for the following volatile aldehydes increased significantly during storage (Figure 1): butanal, 3-methylbutanal, pentanal, hexanal, benzaldehyde and octanal. Butanal, pentanal, hexanal and octanal are all well-known lipid oxidation products. 3-methylbutanal and benzaldehyde have been suggested to originate from non-enzymatic browning reactions (20–22). Butanal, 3-methylbutanal, pentanal and hexanal increased to a greater extent during storage at 20°C and 40°C without exposure to light and at 20°C with exposure to light than at 5°C (Figure 1A-D). Unexpectedly, benzaldehyde and octanal increased most during storage at 20°C without exposure to light followed by 20°C with exposure to light.

In an earlier study, we determined odour detection threshold values for lipid oxidation products, which is the concentration at which the volatile compounds start to affect product odour. However, these were only determined for the volatile compounds that increased during storage in a PSCF. In general, we found that odour detection threshold values in PSCF were above 70 ng/g (5,6). Therefore, volatile compounds present in concentrations below 70 ng/g were not considered to affect product odour when present alone (3-methylbutanal and octanal) in the current study.

The odour detection threshold value determined for butanal was 72 ± 3 ng/g (5,6). In the present study, the concentration was above this level after 3 months storage at 20°C, 20°C with exposure to light or

40°C, and after 6 months at 5°C (Figure 1A). Butanal odour in PSCF has been described as parmesan and sour dishcloth (5,6).

The odour detection threshold value for pentanal (87 ± 5ng/g) was slightly higher compared with butanal (5,6). The concentration was above this level after 3 months at 20°C with exposure to light (at 92 ng/g) or 40°C (at 104 ng/g), and after 6 months at 20°C or 5°C (Figure 1C). Pentanal odour in PSCF has been described as green and milk acidic (5,6). The odour detection threshold value for hexanal has not been determined in PSCFs. Based on the odour detection threshold values obtained for butanal and pentanal, it is estimated to be above 90 ng/g. Hexanal concentrations were above this level after 6 months of storage at all storage conditions. In literature, its odour has been described as fatty, green and fresh (23,24). In addition to aldehydes, a few alcohols and ketones increased as well (Figure 2).

The concentration of 3-methyl-1-butanol was significantly above its odour detection threshold value of 1926 ± 316 ng/g after 6 months of storage. Odour detection threshold values have not been determined for the ketones. However, none of the ketones increased to concentrations above 70 ng/g. Therefore, it is assumed that these ketones did not affect product odour. In a previous study, 3-methyl-1-butanol was described with the odour of glue, rubber, chemical, medicine, cleaning agent (5,6). An expert panel of 3 scientists conducted a DOD sensory evaluation to assess the odour changes, PSCF increased in intensity of chemical and cleaning agent, and scored 3 on the DOD scale after 6 months storage with exposure to light and at 40°C. Since many volatile compounds were present from the beginning of the storage period, they may originate directly from raw materials. Selected raw materials were explored to link volatile compounds in PSCF to those present in raw materials.

Lipid oxidation in selected raw materials

One of the primary functions of a cream is to moisturise and protect the skin so they often contain high levels of lipids, but unsaturated lipids can oxidize and form volatile compounds. Several volatile compounds were present initially in the lipid ingredients and more were generated during accelerated storage at 40°C in the following ingredients: rice bran wax and glycerine (Figure 3A and 3B). PSCF also contained D-panthenol, which was very stable during accelerated storage. Thus, benzaldehyde was the only volatile aldehyde that could be detected and this was not possible until 3 months of storage when 139±9 ng/g was detected (data not shown).

Initially, some raw materials (rice bran wax and glycerine) contained several aldehydes and thus contributed to the initial concentration of all 10 volatile compounds detected in PSCF. Two raw

materials, rice bran wax and glycerine, contained butanal and contributed to the presence of this volatile compound in the freshly produced PSCF. Furthermore, rice bran wax contained 1-pentanol at 262 ng/g and 2-pentanone at 6 ng/g after accelerated storage. Therefore, it is likely that these two raw materials contributed to the development of 1-pentanol and 2-pentanone in PSCF. Moreover, the initial content of pentanal, 3-methylbutanal, 2-hexanone and hexanal in PSCF originated partly from rice bran wax and glycerine. The last aldehyde, benzaldehyde, may originate from D-panthenol (data not shown), rice bran wax and glycerine.

Only low concentrations of volatile compounds were present in glycerine compared with rice bran wax. Glycerine can oxidize to aldehydes such as glyceraldehyde in presence of metal ions and elevated temperature. Overall, 11 different oxidation products that have a three carbon structure have been identified for glycerine. However, the oxidation products can react with other molecules to form compounds with more than three carbons. One proposed mechanism is a reaction between glyceraldehyde and glycerine to form glycerine acetate described by Jungermann and Sonntag (25). Another possibility is simple polymerisation. The purity of glycerine was 99.5%. Moreover, the impurities may also contribute to the volatile compounds developing during accelerated storage.

Rice bran wax (mostly wax esters) mainly contained saturated fatty acids (86%; 16:0,18:0, 20:0, 22:0 and 24:0), in addition to monounsaturated (6.5%; 18:1 n-9) and polyunsaturated fatty acids (3%; 16:3 n-4, 18:2 n-6, 20:3 n-6 and 20:4 n-6). Despite a low concentration of polyunsaturated fatty acids, rice bran wax had significantly higher concentrations of most volatile compounds detected than glyerine because polyunsaturated fatty acids were highly susceptible to auto-oxidation. Auto-oxidation of polyunsaturated fatty acids gives rise to formation of primary oxidation product which can decompose further to secondary oxidation products. One of most likely decomposition pathways is scission. Scission (either α or β) results in a complex mixture of secondary oxdation products inclunding the measured alcohols, ketones and aldehydes (21,22).

The following two raw materials, PMEA and isostearyl isostearate, work as skin conditioners in PSCF. Initially, only hexanal, butanal and pentanal were present in PMEA and isostearyl isostearate (Figure 4), and they may thus partly be responsible for the initial presence of hexanal in PSCF.

Several volatile compounds appeared in the raw materials during the 3 months of storage, but some of these volatile compounds only appeared in PMEA and isostearyl isostearate (2-heptanone, heptanal and nonanal) (Figure 4). However, all 10 volatile compounds that increased during storage in PSCF also appeared and increased in isostearyl isostearate and PMEA, namely butanal, 3-methylbutanal (only isostearyl isostearate), pentanal, 2-pentanone, 1-pentanol (only isostearyl isostearate), 3-methyl-

1-butanol (only isostearyl isostearate), hexanal, 2-hexanone, octanal and benzaldehyde (only PMEA). Therefore, it is likely that PMEA and isostearyl isostearate contributed to the increase observed in PSCF of most of the volatiles. The structure of both PMEA and isostearyl isostearate did not indicate a clear reactive group/site, which can result in the observed volatile compounds. More studies are therefore needed to understand where they originate from. Their presence may be related to impurities present in the raw materials.

The last raw materials included in this study were UV filters. These raw materials were produced with the purpose of being reactive towards pro-oxidants. Three UV filters were investigated: UV benzoate, UV triazine and UV cinnamate. Initially, only a small amount of octanal was present in UV benzoate, and UV triazine did not contain any known oxidation products (Figure 5).

After 3 months of accelerated storage, aldehydes predominantly formed in UV benzoate and UV triazine. Some of the volatile compounds that appeared during the 3 months of storage were not present in PSCF (heptanal and nonanal). UV benzoate and UV triazine generated butanal, 3-methylbutanal (only UV benzoate), pentanal, hexanal, 2-hexanone, octanal and benzaldehyde after 3 months storage.

In contrast to the other two UV filters, UV cinnamate contained substantial amounts of 3-methyl-1-butanol initially, and the concentration of this compound increased further during storage (Figure 5). In addition, UV cinnamate also generated 3-methylbutanal during storage. After three months of accelerated storage, octanal 28 ng/g, pentanal 42 ng/g and benzaldehyde 495 ng/g appeared as well (Figure 5C). Although several aldehydes occurred after three months of storage, their concentrations were low in UV filters compared with the concentrations in humectant, skin texture modifying and skin conditioning raw materials. Therefore, UV benzoate and UV triazine were not explored further. However, the high concentration of 3-methyl-1-butanol generated by UV cinnamate would be expected to impact a finished product odour. A trained sensory panel described 3-methyl-1-butanol as glue, rubber, chemical, medicine and cleaning agent (5,6). Therefore, it is important to understand the route of reactions leading to 3-methyl-1-butanol in order to identify ways to control it.

MacManus-Spencer *et al.* (26) have previously investigated the degradation of octyl p-methoxycinnamate under photolytic conditions and identified 4-methoxybenzaldehyde and 2-ethylhexanol among the products. Two cleavage routes were considered in their work where the alkene in the UV-filter either reacted with water followed by a retro-aldol reaction or a reaction occurred with singlet oxygen to form the aldehydes through an unstable dioxetane (26). The same pathways can be envisioned in our case where UV cinnamate either would form 4-methoxybenzaldehyde and isoamyl

acetate by reaction with water or undergo a cleavage with singlet oxygen to give the corresponding aldehydes (Scheme 1). The addition of water to cinnamates followed by a retro-aldol reaction is a known biosynthetic pathway in the synthesis of plant benzoic acids from cinnamates (27). As a result, it should also be a feasible chemical route although the transformation is probably very slow. The cleavage of olefins by singlet oxygen is well-known (28–30) and the formed isoamyl ester of glyoxylic acid is presumably labile enough to hydrolyse completely under the storage conditions (31). Finally, direct hydrolysis of UV cinnamate to the carboxylic acid and 3-methyl-1-butanol should also be included in the considerations (Scheme 1).

In addition to 3-methyl-1-butanol the degradation of UV cinnamate may thus also form 4-methoxybenzaldehyde and isoamyl acetate which can be used to distinguish between the different pathways. The pH of UV cinnamate was 4.23 initially and decreased slightly to 4.01 after 3 months storage at 40°C. Inspection of the chromatograms from UV cinnamate did indeed reveal the presence of both 4-methoxybenzaldehyde and isoamyl acetate. The retention time was 31.887 for 4-methoxybenzaldehyde and 14.024 for isoamyl acetate and both signals were confirmed by external standards. Notably, the acetate of the alcohol was not detected in the earlier work by MacManus-Spencer *et al.* (26). 4-Methoxybenzaldehyde and isoamyl acetate were both present in UV cinnamate from the beginning of the storage period and their amounts increased further during storage. Although, the two by-products have not been quantified by the use of calibration curves, they appear to be formed in somewhat equal amounts and certainly to a much lesser degree than 3-methyl-1-butanol, which is the main by-product. As a result, 4-methoxybenzaldehyde and 3-methyl-1-butanol cannot be formed by the oxidative cleavage with singlet oxygen since this would give rise to similar amounts of both compounds. Instead, it is very likely that 4-methoxybenzaldehyde and isoamyl acetate are formed by the addition of water and a retro-aldol reaction.

This leaves the direct hydrolysis of the ester as the main pathway for the formation of 3-methyl-1-butanol. It is known that esters can hydrolyse under near neutral conditions, but the reaction is very slow. For ethyl cinnamate the half-life for hydrolysis in water at pH 4.0 and 25 °C is estimated to be about 100 years (32). This number will be higher for UV cinnamate in the present case since the hydrolysis is slower in a non-polar environment. However, the amount of 3-methyl-1-butanol released in Figure 5 only corresponds to about 0.2‰ (w/w) after 3 months storage at 40°C. Therefore it is hypothesised that this is a result of a very slow direct hydrolysis of the ester in the UV-filter under the near neutral conditions.

Linking volatiles in PSCF with those in raw materials

The volatile compounds present in PSCF and raw materials are summarized in Table 3. In brief, the increase observed in butanal in PSCF during storage may mainly originate from isostearyl isostearate, for which the concentration was above odour detection threshold value from the beginning of the storage. However, butanal also developed in rice bran wax, glycerine, PMEA, UV triazine and UV benzoate during storage.

The formation of 3-methylbutanal was related to several raw materials, namely, rice bran wax, glycerine, isostearyl isostearate, UV cinnamate and UV benzoate. The concentration of pentanal increased in all raw materials and the concentration was above odour detection threshold value in rice bran wax, isostearyl isostearate and PMEA after 3 months of storage. Hexanal increased significantly in the PSCF during storage and also increased to high concentrations in rice bran wax and PMEA (more than 150 ng/g). In addition, hexanal was present in glycerine, isostearyl isostearate, UV triazine and UV benzoate in low concentrations (less than 70 ng/g). Benzaldehyde mainly increased in PSCF at 20°C with exposure to light to 112 ng/g after 6 months' storage. It was possible to relate benzaldehyde to all raw materials except isostearyl isostearate. The last aldehyde octanal appeared in all raw materials except glycerine during accelerated storage. Particularly the concentration of octanal increased in rice bran wax. The alcohol 1-pentanol marginally increased in a few materials, rice bran wax and isostearyl isostearate. In contrast, to the low concentration of 1-pentanol and 3-methyl-1-butanol in isostearyl isostearate, 3-methyl-1-butanol was present in high concentration in PSCF from the beginning and throughout the storage period. The RMs shown to generate 3-methyl-1-butanol during storage were UV cinnamate, glycerine and isostearyl isostearate. Lastly, the two ketones, 2-pentanone and 2-hexanone, were present in both PSCF and several raw materials but only in low concentrations.

GSK Toxicology group (2017) has assessed the human safety impact of the volatiles included in this report. At the determined levels these substances do not raise any toxicological concern, neither locally or systemically (33).

Conclusion

This study explored lipid oxidation and oxidative degradation in a topical skin formulation (PSCF) containing high levels of lipid. Some secondary volatile oxidation products were present initially and more were generated during the 6 months of storage. Most notably, 3-methyl-1-butanol was present in a high concentration initially and it increased further during storage. Since the concentration of 3-

methyl-1-butanol was higher than the odour detection threshold value after six months of storage, it was expected to affect product odour after long term storage, generating an increase in the medicinal, chemical/cleaning agent-type odour character. This product was therefore assessed for odour changes (informally vs. a 5°C control sample) and deemed acceptable and typical of a dermocosmetic product, highlighting again the importance of considering the combination effect (of other volatiles present) and the product base odour when interpreting the impact of any lipid oxidation on product odour.

Selected raw materials were explored in order to link volatile compounds affecting the quality in the topical skin formulation to raw material(s). The UV cinnamate filter developed high levels of 3-methyl-1-butanol during storage so was identified as a material to control. A potential pathway leading to 3-methyl-1-butanol was proposed.

Furthermore, well-known lipid oxidation products and non-enzymatic browning products were suggested to originate from rice bran wax in particular because of its unsaturated nature. It was surprising that volatile lipid oxidation products occurred in PMEA and isostearyl isostearate, as these raw materials did not contain reactive sites for oxidation. More studies are needed to explore why volatile compounds appeared.

References

1. Thanonkaew A, Wongyai S, Decker EA, McClements DJ (2015) Formation, antioxidant property and oxidative stability of cold pressed rice bran oil emulsion. J Food Sci Technol 52:6520–6528

2. Poyato C, Thomsen BR, Hermund DB, Ansorena D, Astiasarán I, Jónsdóttir R, Jacobsen C (2017) Antioxidant effect of water and acetone extracts of *Fucus vesiculosus* on oxidative stability of skin care emulsions. Eur J Lipid Sci Technol 119:1600072

3. Malinowska P, Gliszczynska-swiglo A, Szymusiak H (2014) Protective effect of commercial acerola, willow, and rose extracts against oxidation of cosmetic emulsions containing wheat germ oil. Eur J Lipid Sci Technol 116:1553–1562

4. Thomsen BR, Horn AF, Hyldig G, Taylor R, Blenkiron P, Jacobsen C (2017) Investigation of lipid oxidation in high- and low-lipid-containing topical skin formulations. J Am Oil Chem Soc accepted

5. Thomsen BR, Hyldig G, Taylor R, Blenkiron P, Jacobsen C (2017) Odour Detection Threshold Determination of Volatile Compounds in Topical Skin Formulations. Eur J Lipid Sci Technol submitted

6. Thomsen BR, Hyldig G, Taylor R, Gregory J, Blenkiron P, Jacobsen C (2016) Determination of threshold values of lipid oxidation products in skin care products. In: International Symposium on Lipid Oxidation and Antioxidants: PO-18. Porto, Portugal

7. Let MB, Jacobsen C, Meyer AS (2005) Sensory stability and oxidation of fish oil enriched milk is affected by milk storage temperature and oil quality. Int Dairy J 15:173–182

8. Let MB, Jacobsen C, Sørensen ADM, Meyer AS (2007) Homogenization conditions affect the oxidative stability of fish oil enriched milk emulsions: Lipid oxidation. J Agric Food Chem 55:1773–1780

9. McClements DJ (2005) Food Emulsions - Principles, Practices, and Techniques. London, UK: CRC Press:1-26, 167-525

10. McClements DJ, Decker EA (2000) Lipid Oxidation in Oil-in-Water Emulsions: Impact of Molecular Environment on Chemical Reactions in Heterogeneous Food Systems. J Food Sci 65:1270–1282

11. Schultz S, Wagner G, Urban K, Ulrich J (2004) High-pressure homogenization as a process for emulsion formation. Chem Eng Technol 27:361–368

12. Waraho T, Mcclements DJ, Decker EA (2011) Mechanisms of lipid oxidation in food dispersions. Trends Food Sci Technol 22:3–13

13. Heinrichs V, Thum O (2005) Biocatalysis for the production of care specialties. Lipid Technol 17:82–87

14. Garbossa WAC, Maia Campos PMBG (2016) Euterpe oleracea, Matricaria chamomilla, and Camellia sinensis as promising ingredients for development of skin care formulations. Ind Crops Prod 83:1–10

15. Bligh WJ, Dyer EGB (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917

16. Iverson SJ, Lang SL, Cooper MH (2001) Comparison of the Bligh and Dyer and Folch methods for total lipid determination in a broad range of marine tissue. Lipids 36:1283–1287

17. Shantha NC, Decker EA (1994) Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. AOAC Int 77:421–427

18. Thomsen BR, Yesiltas B, Sørensen A-DM, Hermund DB, Glastrup J, Jacobsen C (2016) Comparison of Three Methods for Extraction of Volatile Lipid Oxidation Products from Food Matrices for GC–MS Analysis. J Am Oil Chem Soc 93:929–942

19. Safafar H, Hass MZ, Møller P, Holdt SL, Jacobsen C (2016) High-EPA biomass from Nannochloropsis salina cultivated in a flat-panel photo-bioreactor on a process water-enriched growth medium. Mar Drugs 14:144-153

20. Lu FSH, Nielsen NS, Baron CP, Jacobsen C (2012) Oxidative degradation and non-enzymatic browning due to the interaction between oxidised lipids and primary amine groups in different marine PL emulsions. Food Chem 135:2887–2896

21. Porter NA, Caldwell SE, Mills KA (1995) Mechanisms of free radical oxidation of unsaturated lipids. Lipids 30:277–290

22. Frankel EN (1084) Lipid oxidation: Mechanisms, products and biological significance. J Am Oil Chem Soc 61:1908–1917

23. Buttery RG, Turnbaugh JG, Ling LC (1988) Contribution of volatiles to rice aroma. J Agric Food Chem 36:1006–1009

24. Venkateshwarlu G, Let MB, Meyer AS, Jacobsen C (2004) Chemical and Olfactometric Characterization of Volatile Flavor Compounds in a Fish Oil Enriched Milk Emulsion. J Agric Food Chem 52:311–317

25. Jungermann E, Sonntag NOV (1991) Glycerine: A Key Cosmetic Ingredient. New York, USA: Marcel Dekker

26. MacManus-Spencer LA, Tse ML, Klein JL, Kracunas AE (2011) Aqueous photolysis of the organic ultraviolet filter chemical octyl methoxycinnamate. Environ Sci Technol 45:3931–3937

27. Widhalm JR, Dudareva N (2015) A familiar ring to it: Biosynthesis of plant benzoic acids. Mol Plant 8:83–97

28. Dellagreca M, Iesce MR, Previtera L, Purcaro R, Rubino M, Zarrelli A (2008) Lignans by photo-oxidation of propenyl phenols. Photochem Photobiol Sci 7:28–32

29. Murthy RS, Bio M, You Y (2009) Low energy light-triggered oxidative cleavage of olefins. Tetrahedron Lett 50:1041–1044

30. Matsumoto, Masakatsu Kobayashi H, Matsubara J, Watanabe N, Yamashita S, Oguma D, Kitano Y (1996) Effect of allylic oxygen on the reaction pathways of singlet oxygenation: Selective formation of 1,2-dioxetanes from 1-alkoxymethyl-2-aryl-1-tert-butyl-2-methoxyethylenes. Tetrahedron Lett 43:1319–1323

31. Brachais CH, Huguet J, Bunel C, Brachais L (1999) Identification of small molecules formed from polymethyl glyoxylate degradation in vitro. Polym Degrad Stab 64:243–249

32. Rayne S, Forest K (2016) Carboxylic acid ester hydrolysis rate constants for food and beverage aroma compounds. Flavour Fragr J 31:385–394

33. GSK (2017) Assessment of the human safety impact of the volatiles. GSK, Brentford, UK

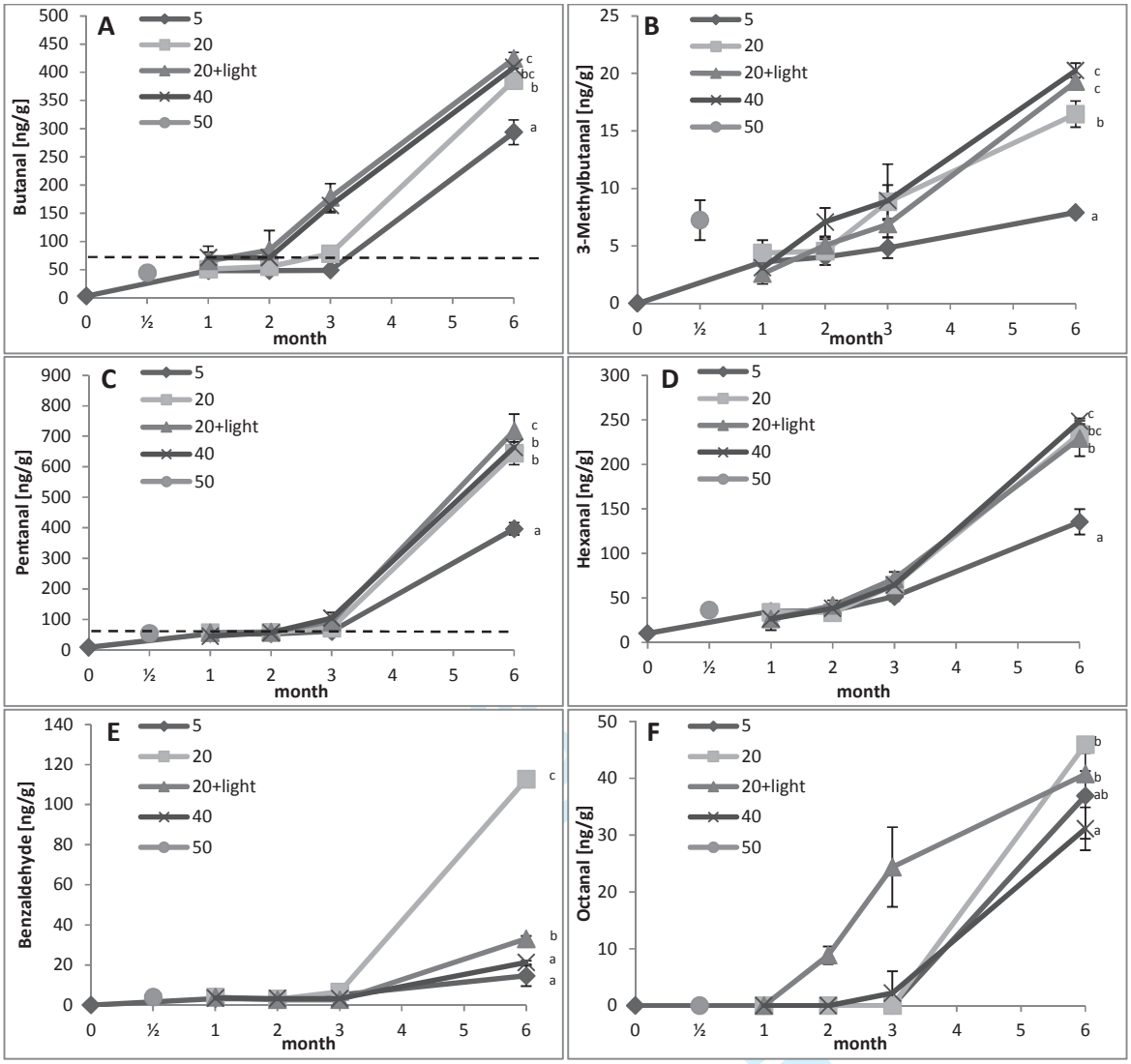


Figure 1. Aldehydes increasing in PSCF during 6 months of storage at 5°C (◆), 20°C (■), 20°C with exposure to light (▲), 40°C (×) and 50°C (●). The dotted line indicates the odor detection threshold value (butanal and pentanal). The development of A) butanal, B) 3-methylbutanal, C) pentanal, D) hexanal, E) benzaldehyde and F) octanal during storage [ng/g]. Results are presented as average +/- SD and N=3. Significance differences at 0.05 level are only marked for the last sampling point.

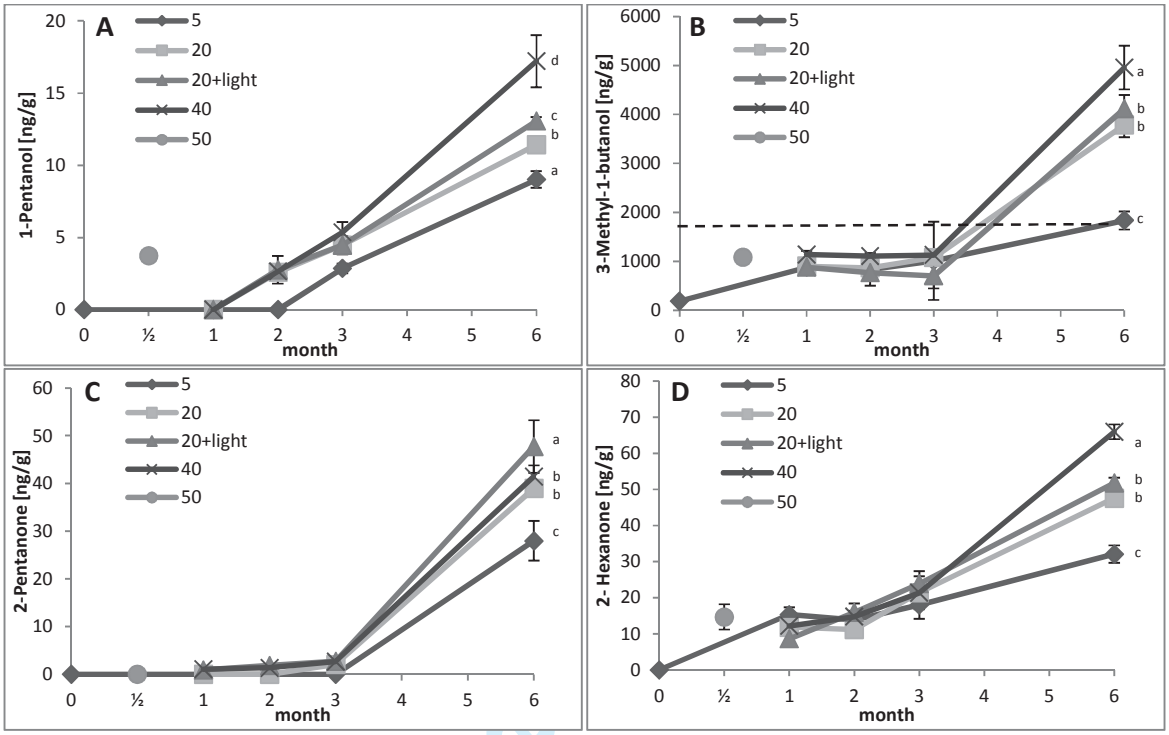


Figure 1. Alcohols and ketones increasing in the PSCF during the 6 months of storage at 5°C (◆), 20°C (■), 20°C with exposure to light (▲), 40°C (×) and 50°C (●). The dotted line is added for the exact threshold value (3-methyl-1-butanol). The development of A) 1-pentanol, B) 3-methyl-1-butanol, C) 2-pentanone, and D) 2-hexanone during storage [ng/g]. Results are presented as average +/- SD and N=3. Significance differences at 0.05 level are only marked for the last sampling point.

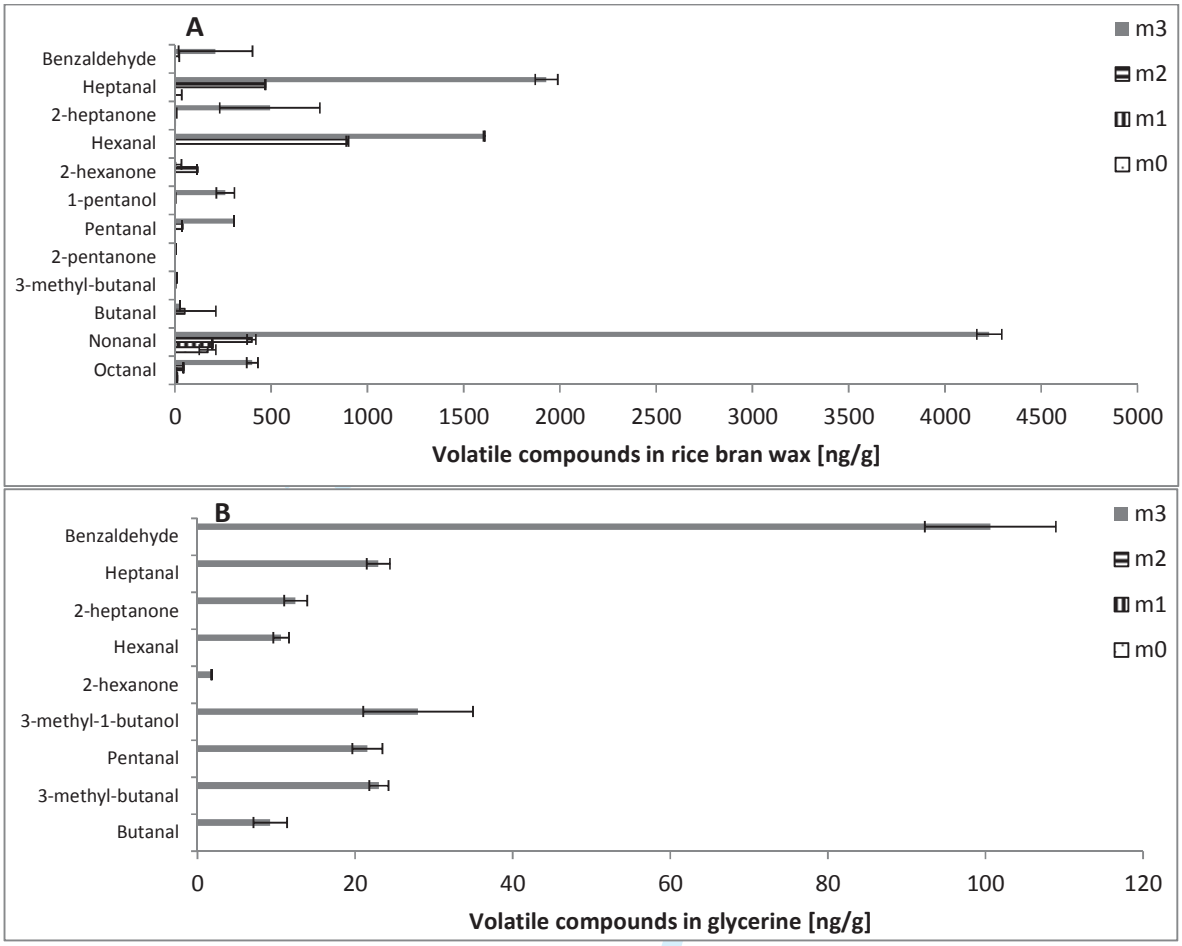


Figure 3. Volatile compounds [ng/g] present in raw materials during the 3-month storage at 40ºC. A) rice bran wax and B) glycerine. Results are presented as average +/- SD and N=3.

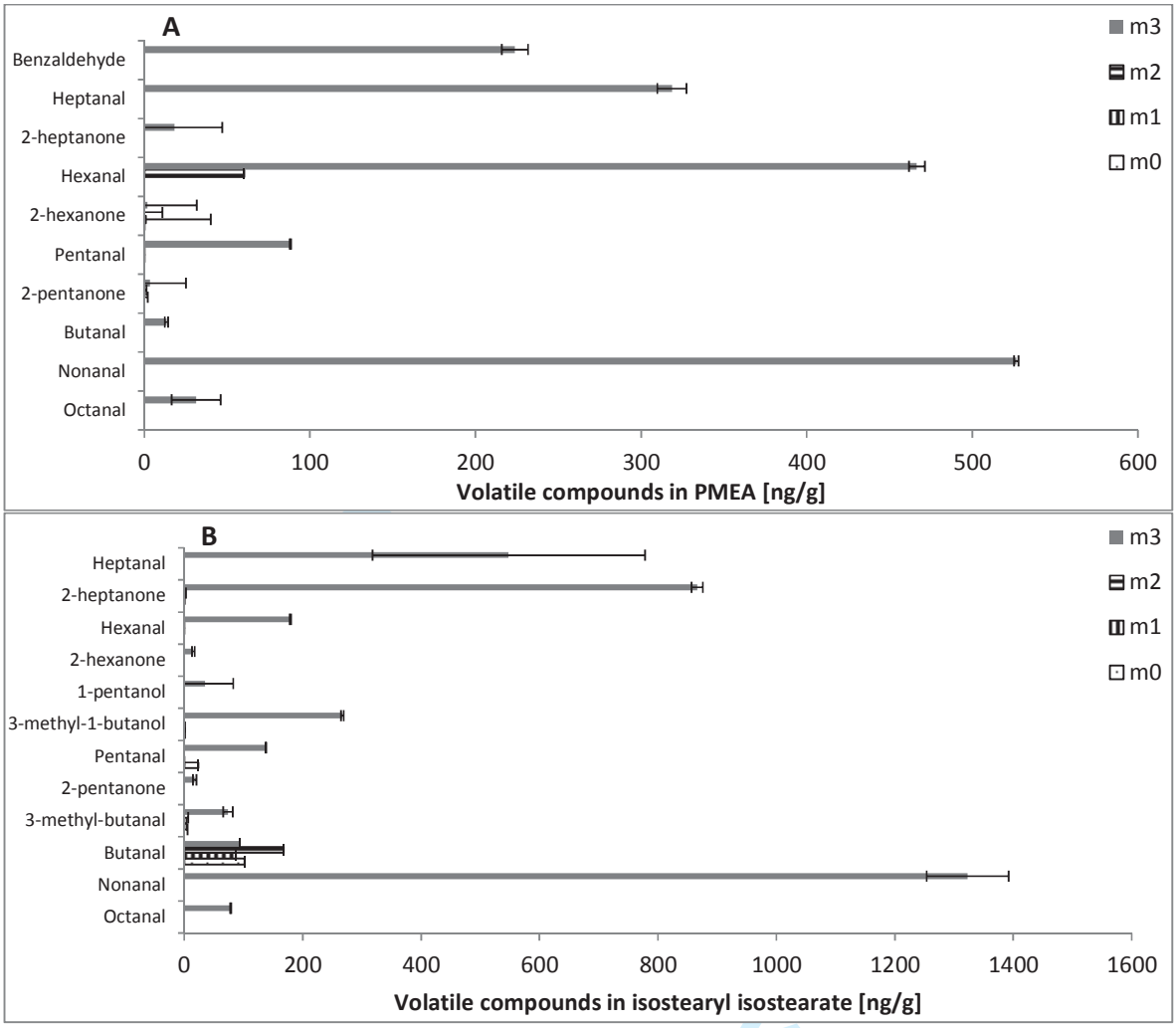


Figure 4. Volatile compounds [ng/g] present in skin texture modifying and skin conditioning raw materials during the 3-month storage at 40ºC. A) PMEA and B) isostearyl isostearate. Results are presented as average +/- SD and N=3.

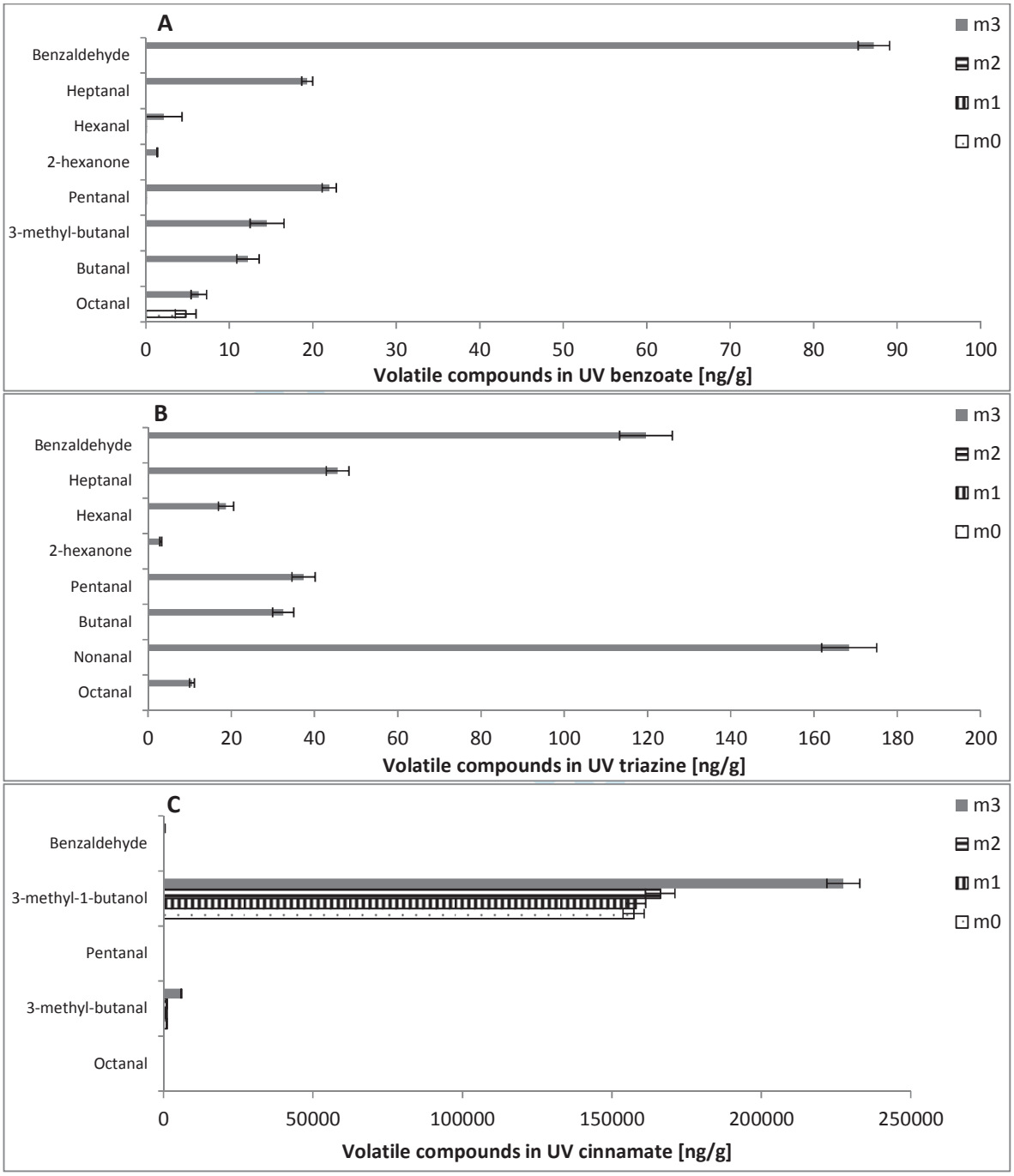
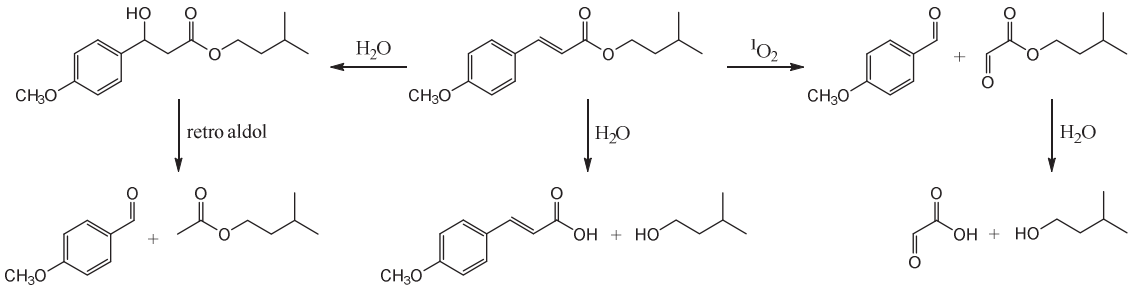


Figure 5. Volatile compounds [ng/g] present in UV filter raw materials during the 3 months of storage at 40ºC. A) UV benzoate, B) UV triazine, and C) UV cinnamate. Results are presented as average +/- SD and N=3.



Scheme 1. Potential pathways for cleavage of UV cinnamate.

Table 1. Sample preparation, collection conditions and water evaporation applied for collection of volatile compounds from PSCF and raw materials.

Samples	Preparation	Collection	Evaporation
PSCF	1 g sample. Incubation at 45°C for 5 min.	50 mL/min at 45°C for 10 min	50 ml/min at 25°C for 22 min.
Rice bran wax Glycerine Isostearyl isostearate UV cinnamate	1 g sample. Incubation at 60°C for 4 min.	50 mL/min at 60°C for 20 min	-
PMEA UV triazine UV benzoate	1 g of sample and water were mixed (1:1). Incubation at 45°C for 5 min.	50 mL/min at 45°C for 10 min	50 ml/min at 25°C for 22 min.

Table 2. Description of difference scale.

DOD Scale	Description of Difference
1	No differences in character or intensity noted
2	Reasonably sure difference exists, though difference may be too subtle to accurately describe
3	Definite difference, can describe difference with reasonable surety
4	Product or material out of expected range. Moderate or large intensity differences or ANY character differences.
5	Outside normal range. Large intensity and/or character differences.

Note: DOD = Degree of Difference

Table 3. Summary of volatile compounds present in both PSCF and raw materials. + = present, ++ = present above threshold value (5,6) **in raw material (only available for butanal, pentanal and 3-methyl-1-butanol), and - = absent.**

Volatile compounds/ Raw material	Butanal	3-methylbutanal	Pentanal	Hexanal	Benzaldehyde	Octanal	1-pentanol	3-methyl-1-butanol	2-pentanone	2-hexanone
Rice bran wax	+	+	++	+	+	+	+	-	+	+
Glycerine	+	+	+	+	+	-	-	+	-	+
Isostearyl isostearate	++	+	++	+	-	+	+	+	+	+
PMEA	+	-	++	+	+	+	-	-	+	+
UV cinnamate	-	+	+	-	+	+	-	++	-	-
UV triazine	+	-	+	+	+	+	-	-	-	+
UV benzoate	+	+	+	+	+	+	-	-	-	+